EC-18 as a Potential Radiomitigator and Radioprotector for the LD70/30 Murine Acute Radiation Syndrome Model

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ABSTRACT

Ionizing radiation can destroy epithelial barriers, increasing vulnerability to subsequent pathogenic infections. Pattern recognition receptors (PRRs) recognize these invading pathogens as well as endogenous danger signals released from dying cells and damaged tissues which then initiate an immune response. Failure to remove these danger signals leads to chronic inflammatory disease, but excessive immune response can further lead to bystander host damage.

EC-18, an oral immunomodulator, facilitates the removal of danger signals induced by exogenous insults and dampens the uncontrolled immune responses by accelerating the intracellular trafficking of PRRs. With this mechanism, we propose that EC-18 can act as both radiomitigator and radioprotector by rapidly removing radiation-induced danger signals, yielding less collateral tissue damage and restoring homeostasis early.

The radiomitigating effect of EC-18 was demonstrated in a total body irradiation LD70/30 survival study in C57BL/6 mice. Mice were administered with PBS (control) or EC-18 daily. To evaluate its optimal dosage, 100, 250, and 375 mg/kg were administered 1-30 day post irradiation. There was a significant increase in survival of each female EC-18- treated group (33%, 50%, and 50%, respectively) as the dosage increased compared to that of the control (4%; p <0.01). The highest dose did not provide any additional survival benefit, indicating that 250 mg/kg is the optimal dosage among the tested doses. A follow-up study is ongoing to optimize the dosing schedule.

In a separate radioprotection study, C57BL/6 mice were treated with PBS or 250 mg/kg of EC-18 24 hr before irradiation, continuing daily up to day 13. There was a significant increase in 30-d survival for the EC-18-treated group compared to the control (45.9% and 12.5%, respectively; $p < 0.001$). The mice in the EC-18-treated group were much more active compared to the control.

Hence, EC-18 possesses significant immune-modulating properties, making it a strong radiation countermeasure capable of both protecting and mitigating the deleterious effects of ionizing radiation.

EXPERIMENTAL DESIGN

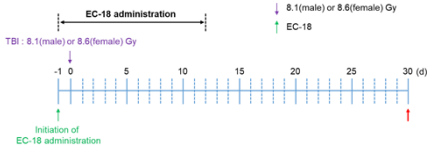
Assessing the Radiomitigating Optimal Dosage of EC-18 for the 30-dose Regimen

- Total body irradiation (TBI) of 680 cGy by X-ray
- 11-12-week-old C57BL/6J female mice (24 mice per group)
- 24-28 hr-delayed treatment of EC-18 daily dosed for 30 days

Group	Rad. Dose (cGy)	Test Article Treatment	Dose Regimen (Days)	Dose Level (mg/kg)
1	680	Vehicle control	1–30	0
2	680	EC-18	1–30	100
3	680	EC-18	1–30	250
4	680	EC-18	1–30	375

Evaluating the Prophylactic Effect of EC-18 for the 15-dose Regimen

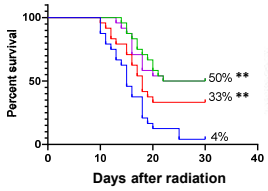
- TBI of 8.1 (male) or 8.6 Gy (female) of ^{60}Co gamma radiation (0.833 Gy/min)
- 12-14-week-old C57BL/6 mice (24 mice per group)
- Dosing regimen: 15-doses
  - Pre-treatment of EC-18 (250 mg/kg) on Days -1 and 0
  - Post-treatment of EC-18 for up to Day 13



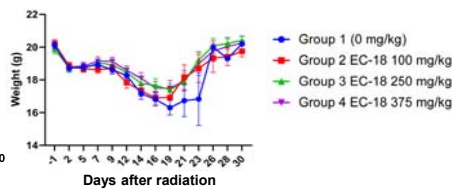
RESULTS

EC-18 as a Radiomitigator for a 24-hr Post-irradiation Treatment

(A1)



(B1)



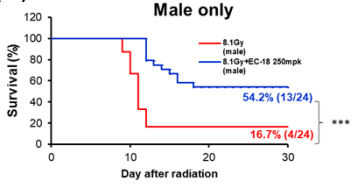
(C1)

Group	Death (%Survival)
1	23 (4 %)
2	16 (33 %) **
3	12 (50 %) **
4	12 (50 %) **

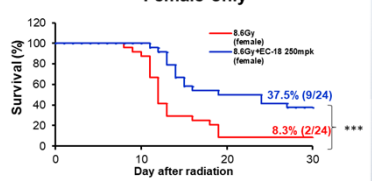
EC-18 may be a potent radiomitigator for the irradiated female C57BL/6J mice, treated 24-hr *post* irradiation: **(A1)** EC-18-treated groups significantly improved animal survivals compared with the vehicle control (PBS) group. **(B1)** There are no significant differences for the changes in mean bodyweights among the treated and the control groups at any point of time during the study. **(C1)** The quantitative results of the percent animal survival for each group; ** indicates $P < 0.05$ compared to the vehicle control group.

EC-18 as a Radioprotector for a 24-hr Pre-irradiation Treatment

(A2)



(B2)

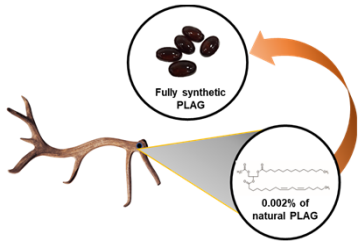


(C2)

Group	Male	Female
	Death (%Survival)	Death (%Survival)
Vehicle Control	20 (16.7 %)	22 (8.3 %)
EC-18 (250 mpk)	11 (54.2 %)***	15 (37.5 %) ***

EC-18 may be a potent radioprotector when treated 24-hr before irradiation: Due to the inherent radiosensitivity, different dosages of radiation were exposed to male **(A2)** and female **(B2)** mice. In both sexes, EC-18-treated group significantly improved animal survivals compared with the vehicle control group and even after the withdrawal of EC-18 (from Day 14), animal survivals were maintained. Moreover, the treatment group appeared to be more active than that of the control group: https://www.dropbox.com/s/afmohktkif06oiy/Behavioral%20Comparison.pptx?dl=0 (On Day 15) **(C2)** The quantitative results of the percent animal survival for each group; *** indicates $P < 0.001$ compared to the vehicle control group.

WHAT IS EC–18?



- Presents as a patient-friendly, soft gelatin capsule for oral administration, with a simple formulation consisting of 500 mg of active pharmaceutical ingredient [1-Palmitoyl-2-Linoleoyl-3-Acetyl-rac-Glycerol (PLAG)] and 1 mg α-tocopherol, as an antioxidant
- PLAG is completely synthetic, lipid-based small molecule (634.99 g/mol), which was first isolated as a natural product from Sika deer antlers
- Subject of two Phase II clinical trials for Chemoradiation therapy-induced Oral Mucositis (CRIOM) and COVID-19 (INDs 135718 and 150887, respectively); Received the Fast Track Designation for CRIOM
- Received approval for pivotal study design to develop a medical countermeasure against Acute Radiation Syndrome (ARS) under the Animal Rule (IND 141545); Received the Orphan Drug Designation for ARS
- Acts as an immunomodulator to rapidly resolve inflammation and restore homeostasis by facilitating the host cell's intrinsic defense responses to remove the pathogenic invaders and danger signals and dampening the pro-inflammatory response and downstream necroptosis pathway.

ACKNOWLEDGMENT

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CONCLUSION

- ❑ EC-18 demonstrated a significant radiomitigating effect in a dose-dependent manner on the female ARS model compared to the vehicle control even upon the 24 hr-delayed treatment.
- ❑ In the dose-range finding study, the highest EC-18 dosage (375 mg/kg) did not provide additional survival benefits and the optimal dosage of EC-18 for radiomitigation of the LD70/30 female C57BL/6J mice turned out to be 250 mg/kg.
- ❑ EC-18 demonstrated a significant radioprotectant effect for both male and female C57BL/6 mice compared with the vehicle control even upon the withdrawal of EC-18 ($p < 0.001$).